STUDIES ON THE HOST-PARASITE RELATIONSHIPS BETWEEN A TREMATODE METACERCARIA *(POSTHODIPLOSTOMUM SP.)* AND A FISH HOST

BY

RALPH DELAVAN LOWELL

A. B., Greenville College, 1938
A. M., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
OF ILLINOIS, 1943

URBANA, ILLINOIS

1943

STUDIES ON THE HOST-PARASITE RELATIONSHIPS BETWEEN A TREMATODE METACERCARIA *(POSTHODIPLOSTOMUM SP.)* AND A FISH HOST

BY

RALPH DELAVAN LOWELL

A. B., Greenville College, 1938
A. M., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY
IN THE GRADUATE SCHOOL OF THE UNIVERSITY
OF ILLINOIS, 1943

URBANA, ILLINOIS

1943

ACKNOWLEDGMENTS

The writer wishes to acknowledge the competent direction of Professor H. J. Van Cleave in this work, and to express sincere appreciation of the time, interest, and encouragement he has unfailingly given.

Acknowledgment is also made to Dr. D. H. Thompson, Dr. G. W. Bennett, and Dr. D. F. Hansen, all of the Illinois State Natural History Survey, who supplied the fish used in the earlier part of this study. Special thanks are due Dr. T. H. Frison, chief of the Natural History Survey, and the Illinois State Conservation Department for permission to use hoop nets.

Correspondence with Dr. M. S. Ferguson of the Rockefeller Institute, Princeton, New Jersey, concerning his own recent findings on the species of parasite studied here and on a closely related strain of the same species has added valuable information to the work.

Finally the writer wishes to thank Mrs. G. W. Hasse who kindly assisted in the typing and proofreading of a major part of the preliminary manuscript.

STUDIES ON THE HOST-PARASITE RELATIONSHIPS BETWEEN A TREMATODE METACERCARIA (*POSTHODIPLOSTOMUM SP.*) AND A FISH HOST.

The present study embodies an attempt to evaluate the effects of a larval trematode *(Posthodiplostomum sp.)* upon the bluegill sunfish *(Lepomis macrochirus)* in whose body an essential part of the life cycle of the worm is passed preceding the attainment of sexual maturity by the marita stage in the intestine of a heron.

Field and laboratory observations have been supplemented by laboratory procedures to determine: (1) the effect of the parasite on the general condition of the fish host, (2) the tissue reactions evoked in the various organs inhabited, and (3) the part played by any immunity or age resistance which might be present.

Taxonomic Status of the Parasite

The strigeid metacercaria considered in this study was originally described by Agersborg (1926) as *Diplostomum vancleavei.* Later it was redescribed by Hughes (1928) under the provisional larval group name Neascus. Ferguson (1937) demonstrated that a form occurring in the blunt-nosed minnow (a metacercaria morphologically indistinguishable from the one in the bluegill) was a stage in the life cycle of *Posthodiplostomum minimum.* With this life cycle known, all similar metacercariae in different fish hosts were given this specific designation. Recently, however, evidence has accumulated to indicate that the metacercaria in bluegills and the metacercaria in blunt-nosed minnows represent physiological strains sufficiently distinct to render uncertain the application of the name *Posthodiplostomum minimum* to the form occurring in the bluegill. Accordingly it is designated *Posthodiplostomum sp.* in this study.

Studies on Condition of Fish Host

This part of the study involved length and weight measurements and parasite counts on 108 bluegills, 32 from Chatauqua Lake on the Illinois River and 76 from the South Pollywogs near Danville, Illinois.

In physiological studies on fish the term "condition" is frequently used. The formulae that have been developed for measuring condition are in reality more accurately described as indices to "plumpness". Two formulae were applied to these fish, $C = \frac{\text{Weight} \times 10^4}{\text{Length}^3}$ (Thompson and Bennett, 1939) and $C = \frac{6 \times \text{Weight} \times 10^4}{\text{Length}^4}$ (Chase, unpublished work, 1940). The first formula gives an undesired correlation between length and condition; the second formula reduces this correlation to less than half that given by the first. Actually such a formula should give no correlation between the length of a fish and its plumpness.

The average index of condition of the Chautauqua Lake bluegills (first formula) was 7.30 and of the Pollywog bluegills 5.73—the latter group showing a definitely poorer condition. When tested by the correlation formula the effect of parasitism on the Pollywog bluegills, as reflected in a lowering of condition, was negligible, being only —0.03. However, the Chautauqua Lake bluegills gave a correlation of —0.12, pointing to a distinct lowering of condition resulting from parasitism. The different results in these two groups suggest that infection of bluegills with this metacercaria may result in a lowering of the condtion of the host by an amount inversely proportional to the loss of condition already present from other causes.

The most significant finding coming out of age analyses of these fish was the fact that three year old and four year old bluegills from the Pollywogs carried almost an identical average number of metacercariae—1343 in the three year olds (range, 75 to 7,000) and 1318 in the four year olds (range, 70 to 5,600). This would indicate that the fish reach a point at about three years of age beyond which continued reinfections do not occur. This suggests the operation of an immunity or age resistance. No evidence was found that metacercariae die, or become resorbed or calcified in the fish host.

Studies on the Histopathology of the Infection in the Fish

Since no cercariae of this worm were secured, studies of histo-

logical changes coincident with experimental penetration, migration, and encystment of the worm could not be made. The information in this section was obtained from fish already naturally infected.

Blood studies (total red cell counts and differential white cell counts) yielded nothing that could be correlated with the parasitism. The twenty-three naturally infected bluegills examined gave the following average differential white cell count: lymphocytes, 56.5%; thrombocytes, 26.0%; neutrophiles, 13.2%; monocytes (mononuclears), 1.5%; "basophiles", 0.9%; eosinophiles, 0.0%; unidentified cells, 1.9%.

Although the fish were not exposed to experimental infection (lack of cercariae), a number of developing metacercariae were found in sections of kidney, liver, and heart in naturally infected fish, and information concerning the encystment of the parasite and changes in host tissue was obtained from these. A total of 61 separate tissues from 18 fish were studied histologically.

The encystment of the metacercaria involves mutual participation of host and parasite. The inner cyst wall is of parasite origin and arises apparently as a hypertrophy of cercarial cuticula; the outer cyst wall is of host origin and is formed by dense fibrous connective tissue or by a flattening of parenchymal cells about the parasite.

The principal changes occurring in host tissues are: (1) displacement and necrosis of parenchyma, (2) cellular proliferation, and (3) replacement of host tissue by parasites.

Head Kidney. The reactions of the head kidney include slight necrosis of cells about the developing metacercaria and also a slight fibroblast infiltration. In moderate cases there is infiltration of leucocytes and reticular cells. In extreme cases there is extensive parenchymal destruction, fibroblast invasion, and congestion.

Kidney. The kidney and accessory kidney show compression and necrosis of tubules about developing worms. Dense fibrous tissue forms the outer cyst wall. In heavy infections, loose fibrous tissue may replace some of the parenchyma.

Liver. The liver ordinarily, even in heavy infections, presents only slight fibrocytic infiltration, the outer cyst wall being mainly composed of flattened parenchymal cells. Some necrosis may occur during the metamorphosis of the parasite.

Ventricle. On the surface of the ventricle each metacercaria is enclosed in a thin layer of dense fibrous tissue and the cysts, which may be several layers deep, are attached to each other and to the ventricle by loosely organized connective tissue in which fibroblasts, reticular cells, histiocytes, lymphocytes, and granulocytes are found.

Studies on Immunity in the Fish Host

Precipitin tests for naturally and artificially acquired immunity were made on bluegills already carrying natural infections. No uninfected fish were obtained.

Of 47 tests for naturally acquired immunity 23 were positive, 6 negative, and 18 inconclusive due to irregular precipitation or bacterial contamination occurring when blood was taken from the gills. The titers varied from 1:1 to 1:280 but showed no correlation with the degree of infection.

Of 24 fish given injections of ground worm material, only four survived long enough for the second precipitin test to be made. Two of the first three gave positive tests before injection (the other was inconclusive), but all three gave negative tests two weeks after the first injection. The fourth gave a negative test before injection and a positive test a month later. There is some evidence to indicate that the temperature of the water (12° C.) in which the fish were kept may have had a bearing on the results. Gee, of the Wisconsin Conservation Department, has found that cold water retards the development of antibodies.

The above data do not give consistent evidence for the existence of a precipitin in the blood of naturally infected bluegills, although the results suggest that such an antibody may be present. The explanation of the levelling off of the average number of metacercariae at about three years of age is probably to be found primarily in an age resistance.

Summary

1. Infection of bluegills with metacercariae of *Posthodiplostomum sp.* may result in lowering the condition of the host by an amount inversely proportional to the loss of condition already present from other causes.

2. The three year old and four year old bluegills from the Pollywogs carried nearly the same average number of metacercariae, suggesting the presence of an age resistance or an immunity.

3. Blood studies yielded nothing that could be correlated with the parasitism. Information is given concerning the types and proportions of white blood cells in the bluegill.

4. The cyst of the metacercaria is composed of two layers. The outer layer is of host origin, consisting of dense fibrous tissue or flattened parenchymal cells; the inner layer is of parasite origin and is derived from cercarial cuticula.

5. Changes in host tissue may be grouped under three heads: (1) displacement and necrosis of parenchyma, (2) cellular proliferation, and (3) replacement of host tissue by parasites.

6. Immunity studies were inconclusive, only justifying the statement that a precipitin may be present in naturally infected bluegills. Age resistance is the most favored explanation for the levelling off of the average number of metacercariae in bluegills three years of age and older.

VITA

Ralph Delavan Lowell was born in Cortland, New York, July 6, 1917. He attended elementary schools in Greenville, Illinois, San Diego and Santa Ana, California; and Spring Arbor, Michigan. He was graduated from high school in 1934 and junior college in 1936 from Spring Arbor Seminary and Junior College, Spring Arbor, Michigan. His college work was completed at Greenville College, Greenville, Illinois, in 1938.

On graduation from college he received a graduate scholarship to the University of Illinois, but this was resigned in the Fall of that year in favor of an assistantship. He received the M.A. degree in 1939.

Since that time he has continued his study toward the doctorate at the University of Illinois with the exception of a seven and a half month interim (July 27, 1942 to March 13, 1943) spent in Signal Corps training in Chicago as an enlisted reservist in the United States Army.

During the Summer of 1940 nine weeks were spent at Friday Harbor, Washington, studying the marine fauna of that region.

He has been on part-time appointment as assistant in zoology during his time in residence at the University of Illinois. He is a member of Phi Sigma biological society, Phi Kappa Phi, and Sigma Xi.